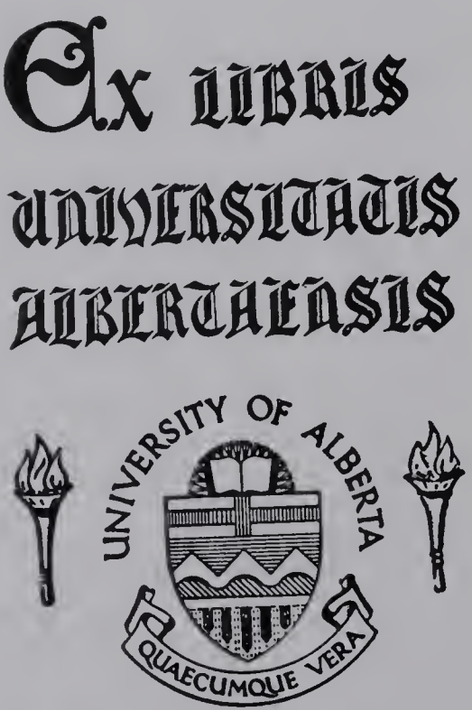

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MINERAL EQUILIBRIA IN MEAT

by

THERESA T. BALDWIN, B.SC.

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ABSTRACT

This investigation was concerned with the concentrations of certain inorganic elements in meat, their relative degree of binding and the relationship of muscle components to changes in tenderness. Concentrations of calcium, magnesium, inorganic phosphorus, and citric acid were determined.

The addition of polyphosphates significantly increased the tenderness of meat. The pH values of phosphate-treated samples and citrate-treated samples were significantly different from the untreated samples.

More than half of the total calcium, magnesium, phosphorus and citric acid was present in the soluble fraction; the remainder was bound to the protein and structural components of the muscle. Addition of citrate and phosphate resulted in the removal of calcium from its bound state with the insoluble protein. The distribution of magnesium, however, was unaffected by the presence of added citrate and phosphates.

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MINERAL EQUILIBRIA IN MEAT

INTRODUCTION

Recent studies have indicated that the major quality characteristic in meats which is desired by the consumer is that of tenderness. The factors which are responsible for differences in tenderness have not been investigated sufficiently to establish a definite picture. It is generally agreed that the following factors play an important role in determining meat quality: age, sex and breed of the animal, feeding practices, aging, resolution of rigor, enzyme action, acidity, solubility of collagen, muscle activity, fat content, the presence of inorganic salts and the method of cooking. The underlying causes of how each of these variables affects meat quality are far from being completely understood. That this should be so, despite intensive research, is not surprising in view of the variability in the composition of the meat and its complex nature.

Reports of several studies which have appeared in the literature seem to point to the possibility that inorganic ion-protein relationships and mineral equilibria in general are of importance in determining the quality of meat. It appears, from studies of many authors working in the field of the biochemistry of muscular contraction, that this involves a well ordered series of reactions. They comprise hydration, ionic binding involving adenosinetriphosphate (ATP), magnesium and potassium ions, and the

reaction between actin and myosin to form actomyosin. Ion-protein interactions have long been of interest to those concerned with milk and cheese processing. Accordingly there appeared to be a need for an investigation in which meat, subjected to possible changes in ionic balance, is analysed in detail and an attempt made to relate the mineral equilibria to some physical properties of meat. This was the object of the present investigation. The determination of tissue components responsible for variations in the tenderness of meat is of considerable practical importance and therefore was also considered in this investigation.

General Principles

This study requires as a first consideration a knowledge of the characteristics of both the meat and some of its constituents - water, ions and proteins, as well as the interactions which these constituents may undergo.

Food and Drug Directorate defines meat as the clean dressed flesh exclusive of lips, snouts and ears of animals healthy at the time of slaughter, and includes the heart, tongue, diaphragm, oesophagus and the skeletal musculature with attendent tissues. It is primarily composed of lean muscle and connective tissue, this being made up of approximately 75% water and 20% protein, the remaining 5% consisting of fat, carbohydrates, minerals and non-protein water extractives.

(a) The Proteins of Meat The muscle proteins are the principle solids of meat. The major proteins in muscle are myosin and actin. Myosin comprises the greater part representing approximately 38% of the total. It possesses a relatively high negative charge and

shows a strong affinity for the divalent cations of calcium and magnesium. Actin, on the other hand, represents about 13% of the total muscle protein and exists in two forms: as a monomer (Globular Actin or G-Actin) and as a polymer (F-Actin). In the presence of ions and a small amount of ATP, G-Actin polymerizes to F-Actin. F-Actin may combine with myosin to form a complex called actomyosin or Myosin-B which is the contractile element of the muscle.

The contractile proteins of muscle fibers are separated or surrounded by layers of connective tissue or stroma which constitute about 10 - 15% of the muscle proteins with the remainder consisting of albumins, globulins and respiratory proteins.

(b) The Water of Meat Meat contains about 75% water which is obviously immobilized in some manner. The immobilization is probably due partially to a combination of water molecules with other muscle constituents and partially to occlusion of water between a lattice of fibers and membranes.

(c) The Inorganic Constituents of Meat Less than 5% of the total constituents in edible muscle tissue are inorganic in nature. These minerals are partially in an ionic form and partially bound to the protein molecules. This inorganic material is essentially composed of the elements calcium, phosphorus, potassium, magnesium and iron. Fresh beef muscle is generally considered to have between 3 - 12 mg/100 gm calcium, 109 - 220 mg/100g phosphorus, 15 - 30 mg/100g magnesium,

450 mg/100g potassium and 2.5 mg/100g iron. Trace amounts of other elements are also found.

REVIEW OF THE LITERATURE

A. FUNDAMENTAL BIOCHEMICAL FACTORS AFFECTING MINERAL EQUILIBRIA

1. Post-Mortem Biochemical Changes

The most prominent physical change which occurs soon after the death of an animal is a hardening of the muscles. These become hard, inflexible, contracted and tough - a condition known as rigor-mortis. If the animal is held or 'aged' for several days after death the muscles again become soft, pliable, relaxed and tender. Various workers have attempted to correlate these physical changes with mineral equilibria in the tissue.

Recently Hamm (1962) concluded that immediately after death muscle ATP is not free but bound with alkaline earth metals (Ca, Mg, Zn) to muscle proteins. During the process of rigor-mortis enzymatic dephosphorylation releases this nucleotide. Some of the released ions are bound immediately by the negatively charged groups of the muscle protein and therefore are incorporated into the structure of the tissue, causing a dehydration.

Arnold et al. (1956) found that during post-mortem aging of beef, sodium and calcium ions were continuously released by the muscle proteins, and potassium ions were absorbed during the first 24 hours. Magnesium ions were released during the first 24 hours, and also between the sixth and thirteenth days after which their

Review of the Literature

A. Biochemical Changes Affecting Muscle Extensibility

1. Post-Mortem Biochemical Changes

The most prominent physical change which occurs post mortem is the death of an animal is a hardening of the muscles. Lawrie [1966], following, contracture and rigor - a condition known as rigor-mortis. If the animal is held or 'aged' for several days after death the muscles again become soft, pliable, relaxed and tender. Various workers have attempted to correlate these physical changes with chemical equilibria in the tissue.

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Arnold et al. (1960) found that during post-mortem aging of beef, sodium and calcium ions were continuously released by the muscle proteins, and potassium ions were absorbed during the first 24 hours. Magnesium ions were released during the first 24 hours, and also between the fresh and frozen/th data after which there

release decreased. The total cationic shift resulted in greater hydration, increased protein charge and improved tenderness.

Initially Wierbicki et al. (1954) presented evidence suggesting that increases in tenderness with post-mortem age may be related to (a) the dissociation of actomyosin or some similar protein changes which increase protein extractability, and (b) the redistribution of ions within muscle thus causing increased hydration and tenderness. However in a later publication Wierbicki et al. (1956) reported that no evidence could be found for the dissociation of actomyosin during the aging process. He stated that the post-mortem tenderization may be due to certain unspecified protein-protein or ion-protein interactions.

Webb (1960) indicated that increased tenderness during aging of beef was associated with water-extractable minerals as well as changes in pH, water holding capacity and water extractable nitrogen.

In his study of poultry leg and breast meat van den Berg (1964) showed that water holding and ion-binding properties changed markedly during the first 1 - 2 days of storage. Subsequent changes were generally small.

Fujimaki et al. (1960) reported that the content of alkaline earth metals in actin during aging of meats was at a maximum immediately after slaughter and at a minimum after two days of aging. The ratio of calcium to other alkaline earth metals was high immediately

after slaughter but became low during aging. He, therefore, concluded that in meats actin is present both in free and bound forms existing in the former immediately after slaughter. However after two days of aging, it combines with myosin to form actomyosin.

It is likely that alkaline earth metals play an important role during the aging of meat and the resolution of rigor.

2. Hydrogen Ion Concentration

Of particular importance is the effect of hydrogen ion concentration on mineral equilibria in meat. However, very little research has been done in this field.

There is agreement that one of the principle changes which occurs in muscle between death and the onset of rigor-mortis involves an increase in the lactic acid content and therefore a decrease in pH. Bate-Smith (1948) reported that in beef a pH between 5.4 and 6.0 may normally be expected after aging, but lower or higher values may occasionally be encountered. He further reported that within these limits of variation pH has a marked effect on both the physical and biological properties of meat. Near the upper range of pH of post-mortem muscle the meat is dark in color, slimy and yielding to the touch. The juice is not readily extracted and the electrical resistance of the meat is so high that salt will not readily penetrate. An increase in resistance with increasing pH was regarded as being due to the swelling of the fibers with a resulting narrowing of the channels

through which ions could freely move.

Hamm (1956) discussed the post-mortem changes in the cation binding capacity of meat. These changes involved gradual decrease especially in the concentration of Mg and Zn ions and to a lesser extent in Ca ions. This may be due to changes in hydrogen ion concentration. In a later report (1957) he confirmed this statement indicating that pH determines the amount and direction of calcium and zinc ion effects.

In an article on the minerals of mammalian muscle Hamm (1959b) indicated the binding of ions to the proteins of rigor muscle at pH 5.5 and 7.0 as shown in Table 1.

Wismer-Pederson (1965) studied the effect of pH and divalent cations, such as magnesium and calcium, on the rehydration of freeze-dried meat. He reported an increased hydration capacity with higher pH values whether the above cations were present or not.

In his study on ion movement across cell membranes Gilbert (1961) pointed out that changes in pH may influence the transport mechanisms of both calcium and magnesium ions. Both calcium and magnesium ions contributed to the buffering of pH. The lowering of pH to 2.2 was found to release some 75% of intracellular magnesium. On the other hand, it appeared that a higher pH of 10.0 significantly increased the binding of calcium. He further stated that the failure of these two divalent ions to exhibit quantitatively equivalent

TABLE 1. CONTENTS OF MAGNESIUM, CALCIUM, ZINC AND IRON IN THE STRUCTURAL PROTEINS OF BEEF MUSCLE*[1]

1 g protein has bound (micromoles):

pH	Mg	Ca	Zn	Fe
5.5	6.7±0.6	4.0±1.0	2.0±0.1	1.0±0.0
7.0	24.4±2.4	6.5±0.7	1.9±0.1	1.1±0.0

* Post-mortem storage for 5 days at 2°C.

1 From Hamm (1959b)

shifts with changes in pH possibly relates in part to the different binding properties at the binding sites for these ions.

We may therefore conclude that it is possible that differences in muscle pH may cause varied results depending on the interrelationship of constituents within an individual carcass.

3. Hydration

The divalent cations present in meat have an important influence on the water holding capacity of the meat in spite of their relatively low concentration.

Wierbicki et al. (1956) reported that changes in tenderness seemed to coincide with those of juice loss, pH and water extractability. It appeared from these findings that tenderness is closely related to the degree of hydration of meat proteins. He also reported that the juice loss of meat on cooking was profoundly affected by hydrogen ion concentration. In addition, other factors such as the mineral ions present, appeared to control protein hydration.

Arnold et al. (1956) studied the relationship of the degree of hydration of muscle protein to ionic shifts during post-mortem aging. The total cationic shift was seen as a movement of cations onto the meat proteins. This resulted in an increased charge on the meat proteins allowing greater hydration and improved tenderness. It was concluded that the importance of cations to hydration and tenderness of meat was not due to the total amount of each

cation present but rather to their combined effect and to the movement of such ions during post-mortem aging.

It is known that polyvalent cations may decrease the hydration of proteins, linking together the peptide chains by forming cross-linkages (Haurowitz, 1963). Bozler (1955) found that a partial extraction of calcium by ethylene diamine tetra-acetate (EDTA), and of magnesium by polyphosphate increased the swelling capacity of muscle tissue. Hamm (1955a, 1958b) showed that a partial exchange of bound muscle calcium and zinc for sodium or potassium at pH7, by means of exchange resins, increased the water holding capacity of beef muscle. However, bound magnesium and iron were not exchangeable under these conditions. It follows therefore, that the elimination of calcium and perhaps of zinc, is responsible for the hydration effect produced. Nevertheless, some evidence exists that not only bound calcium but also bound magnesium decreases the hydration of muscle (Hamm, 1959a). Later this author (Hamm, 1962a) reported that the removal of calcium or magnesium by cation exchange, or complexing, increased the hydration of meat. Hamm (1960) reviewed all aspects of meat hydration in an article entitled "Biochemistry of Meat Hydration".

Fujimaki et al. (1961) reported that there are intimate relationships between the water holding capacity and the content of alkaline earth metals in meat. He ascertained that the release of such metals from meat proteins, especially actomyosin, increased the

water holding capacity of the meat.

Swift and Berman (1950), in their study on the factors affecting the water retention of beef, reported a direct, highly significant correlation between water retention and zinc content. In contrast, an inverse relationship was found between water retention and either calcium or magnesium content. It appeared that zinc differs in some important aspect from the alkaline earth metals. The possibility is indicated that zinc may participate in determining pH as a component or an activator of an enzyme system. Prior to the above report the action of zinc had been considered to be similar to that of calcium in that its binding to the structural proteins of meat had been assumed to have an adverse effect on water retention (Hamm, 1956). Swift and Berman (1959) reported that zinc may have this tendency, however, the parallelism between zinc content and pH in its relationship to water retention must be considered.

Deatherage (1956) summarized all the above factors as follows:

"It was established that tenderness of meat was related to the ability of meat proteins to hold water. This property of meat could be modified by altering the ionic atmosphere in the muscle (meat) proteins. Furthermore, it was established that a major factor in post-mortem tenderization of meat is the randomization (redistribution) of ions which occurs post mortem in

muscle in a manner to cause the muscle proteins to hold more water and thus to shrink less on cooking, resulting in a softer coagulum of proteins - tender meat".

Factors concerning the hydration of meat which contains various ionic additives will be discussed in a later section.

4. Protein Interactions and Ion Association

Because charged centers on a protein molecule attract small cations and anions, alkaline earth metals may be bound directly to the actual protein molecules. However, the extent of binding varies greatly with the nature of the particular protein and cation involved. van Leeuwen (1964) distinguished two types of cationic binding by protein:

(a) the complex type; here the cation reacts with specific groups on the protein and no longer contributes to the ionic activity of the surrounding fluid

(b) the salt type; here the cationic charge statistically balances the negative charge of the protein without losing its ionic properties.

Bárány et al. (1957) reported that not only the peptide chains within the same protein component (e.g. myosin, actin) of muscle are linked together by alkaline earth metals but also inter-linkage of different proteins may occur.

The bound alkaline earth metal ions of the structural proteins were studied in detail by Hasselbach (1957). According to his investigations the "insoluble protein structures", the main mass of which is made up by the myofibrils, contain 60% of the total calcium in muscle and 10% of the total magnesium. In the myofibril preparations about 50% of the calcium is firmly bound. This was confirmed by Mühlrad and coworkers (1962) who further stated that in the formation of a linkage between myosin or myosin-B (actomyosin) to the fibrils and calcium, two kinds of binding sites participate - a weak and a strong site, the latter specifically showing a preference for calcium rather than magnesium ions.

Ghosh and Mihályi (1952) have stated that the affinity of myosin for alkaline earth cations is very pronounced. Nanninga (1957) also reported that magnesium and calcium are strongly bound to light and heavy meromyosin above pH 4.5, the binding increasing with pH and free ion concentration.

Actin, the other principal contractile protein has also been reported to have a great affinity for specific cations. Chrambach et al. (1961) reported that G-Actin contains approximately one metal ion per protein molecule, the majority of such ions being largely accounted for by calcium. The magnesium and zinc content calculated by independant methods seems negligible.

Martonosi et al. (1964) reported that the binding of divalent cations to actin occurs at two types of binding sites. In

his study on cations and actin Katz (1963) stated that calcium and magnesium have specific effects on the configuration of G-Actin, the former acting to favour a "closed" configuration while substitution by the latter produces an "open" configuration. Bárány and coworkers (1962) reported that the bound calcium of G-Actin is rapidly and completely exchangeable. This reaction is reversible. The bound calcium of F-Actin is very strongly attached and not easily removed. He further reported that whenever bound calcium is removed, actin loses its ability to polymerize and form actomyosin.

Fujimaki et al. (1960) stated that when actin and myosin join to form actomyosin, actin loses its alkaline earth metal content.

A general agreement exists that the binding of actin to myosin agrees with a weight ratio of approximately four myosin to one actin (Spicer and Gergely (1951) and Nanninga (1964)).

An understanding of the interaction of myosin with polyphosphates is also important in the study of muscle properties. Yausi et al. (1964a) distinguished between two types of binding with the protein - one in which polyphosphates of comparatively low molecular weight (pyrophosphates or tripolyphosphates) react as a salt with the salt free myosin-B. Their affinity for myosin-B is greatly enhanced in the presence of high salt concentration and divalent cations. The second type involves polyphosphates of a

high degree of polymerization (e.g. hexametaphosphate) which bind directly with salt free myosin-B but their binding is somewhat inhibited at high salt concentration and in the presence of divalent cations. In a later study Yausi (1964b) further reported that the basic function of the inorganic polyphosphates which affect the binding properties of meat is to cause a shift to the right in the following equilibrium:

$$\text{actomyosin} \rightleftharpoons \text{actin} + \text{myosin}$$

Fujimaki (1965) agreed with this statement, reporting that a residual amount of ATP at rigor would mean a decrease in the interaction between actin and myosin in actomyosin.

Lyons and Siebenthal (1966) studied the binding of condensed phosphates to proteins in general. They assumed that any interaction between a phosphate and a protein molecule involves the same sites on the phosphate as are engaged in the formation of soluble calcium complexes with the phosphate. Therefore, any reduction in sequestering power in the presence of protein may be attributed to protein-phosphate interactions. Their results showed that there is clearly an effect dependant on the chain length of the polyphosphate. In the case of pyrophosphate one interaction with a protein site may eliminate almost totally its complexing power for calcium. However in the case of tripolyphosphate, a pyrophosphate "tail" remains which may be able to form moderately stable calcium complexes. In the case of long chain polyphosphates there are probably multiple interactions with the proteins.

Comparing this theory with the results obtained by Yausi (1964a) it may be postulated that the inability of highly polmerized polyphosphate to bind salt-free myosin-B in the presence of divalent cations may be due to the competitive reaction between the cation and the protein to bind the polyphosphate, the divalent cation-polyphosphate complex being the more stable.

In meat the alkaline earth metals may exist in a free ionic form, bound to other ionic constituents as salts, or bound to other molecules forming a complex species.

The affinity of citrate for calcium ions is a very interesting example of complex formation. The divalent calcium ion "fits" between two of the three dissociated carboxyl groups of citrate and is so firmly incorporated into the resulting species that both the two carboxyl groups and the calcium ion cease to contribute to the ionic strength of the solution (Hastings et al. (1934)). Walser (1960) reported that calcium citrate was a chelate compound. He also pointed out that the possibility of ion pairs exists, in which ions such as that of calcium may be electrostatically paired with multivalent ions. Calcium ferrocyanide, calcium sulfate and calcium phosphate are considered to be examples of such ion pairs.

He further reported that the ion pairs themselves may be bound at the binding sites of the muscle protein. Calcium and phosphate ions associated electrostatically with protein may also

become associated with one another. Thus calcium ferrocyanide, calcium sulfate and calcium phosphate ($CaHPO_4$) cause an increase in protein bound calcium and a reduction in free calcium concentration. However, citrate forms a chelate complex with calcium therefore with increasing citrate concentration, calcium ion concentration and protein bound calcium both decline.

B. INFLUENCE OF ADDED COMPOUNDS ON THE MINERAL EQUILIBRIA IN MEAT

Thus far we have been concerned primarily with those constituents which occur naturally in meat. A variety of inorganic compounds may be added to some meat products to improve their quality. Salts such as chlorides, phosphates and citrates have been used.

1. Addition of Chlorides

The effect of adding the chlorides of sodium, potassium, calcium and magnesium to meat has been studied by Wierbicki *et al.* (1957b). All the above chlorides when added prior to heating increase the water holding capacity of cooked meat, proteins with magnesium chloride showing the most pronounced effect. A combination of sodium and magnesium chlorides showed the greatest effect in promoting water-holding capacity and the beef being studied was made more tender.

Magnesium and calcium chlorides lower the pH of raw

meat and tend to decrease the water holding capacity of unheated meat proteins (Hamm, 1956). Apparently this is not so in heated meat where a number of complex reactions are taking place. Wierbicki (1957a) also reported that the addition of sodium chloride increased the absorption of both potassium and magnesium ions with almost no effect on calcium. The significance of these ionic shifts are not fully understood.

The water binding capacity of fresh pork was studied by Sherman (1961). The addition of sodium chloride improves fluid retention. The action of sodium chloride and other neutral salts is dependant on the degree of ion absorption, with the anions being absorbed preferentially. In a later paper Sherman (1962) described this theory in more detail. Ion absorption opens up the meat protein structure by reducing the internal forces of attraction, thus permitting water to be absorbed.

Hamm and coworkers (1955b, 1958a) have done a considerable amount of work in this field. The addition of sodium chloride is reported to have a direct influence on protein hydration. Meat salted on the day of slaughter possesses a high water binding capacity which it retains for at least seven days. Calcium is more strongly bound in slaughter-warm salted meat than in untreated meat. The normal post-mortem decline of dissociated acid groups is inhibited by sodium chloride addition. In a later publication Hamm (1960) stated that the decisive reaction involved in the

salting of meat is the binding of the anion to the protein. Niinivaara and Pohja (1954) reported another theory on the action of sodium chloride. The presence of salt which caused an increased water binding was thought to depend on the changes in the isoelectric point of the meat proteins.

2. Addition of Phosphates

The addition of phosphates to meat products has received a great deal of attention in the last few years. The polyphosphates are used commercially to improve water holding capacity and binding properties of meat.

The swelling effect on meat of inorganic pyrophosphate or tripolyphosphate (Bendall, 1954, 1958) can be traced back to their ability to split actomyosin into its component proteins, actin and myosin, resulting in the uptake of water. Therefore, the effective polyphosphates should have chemical structures similar to that of ATP. Fukazawa (1961) also reported that the state of protein in the muscle is important in determining the effect of phosphate on the water binding quality of sausage. If the myofibrillar protein is in the native state, the action of phosphate is correlated with the amount of protein extracted from these fibrils. However, in the denatured state, the effect has been correlated with the dissociation of actomyosin and the increase in the myosin content. This explanation is based on the action

of pyrophosphate. Swift and Ellis (1956) also showed in their work that pyrophosphates dissolve protein, especially actomyosin, to an extent affected by ionic strength and pH. On the other hand, Hamm (1958a) showed that phosphates exercise a strong hydrolytic action on actomyosin. He further stated that swelling and hydration of muscle proteins is important in determining meat quality.

The changes in pH due to additions of phosphate have also been studied. Hamm (1955b) reported that the actions of phosphate on meat containing salt is determined by both increase in pH and a "salt effect". Swift and Ellis (1956) showed that the effectiveness of pyrophosphate treatments was primarily related to the ionic strength and pH of the solution applied. Hellendorn (1962) reported that at pH values below 5.5, pyrophosphate and tripolyphosphate exerted a depressant effect on the water binding of uncooked meat. In the heated samples within the normal pH range of 6.0 - 6.5 pyrophosphate and tripolyphosphate had a marked specific activity, the former being slightly superior.

Other explanations for the effect of phosphates on meat quality have been described. Hamm (1960) suggested that the effect of polyphosphates and citrates is not an immediate interaction between the anion and the protein but a more indirect influence. He postulated that the influence of these salts on the water holding capacity of meat is due to an elimination of the

alkaline earth metals bound to the structural muscle proteins by precipitation, or the formation of undissociated complexes. Only the free ions, not those bound in complexes are able to increase the muscle hydration. For example calcium tripolyphosphate is not active. In contrast to this report, Hellendorn (1962) reported that the elimination of calcium from the meat has nothing to do with the water binding capacity. The specific activity of pyrophosphate, for example, cannot be explained in terms of its ability to complex calcium ions. This view is also supported by Sherman (1961) who stated that tetrasodium pyrophosphate or alkaline polyphosphate are particularly effective in fluid retention, but that this cannot be attributed to their ability to complex calcium and magnesium ions. His theory was explained in a later paper (1962) where he stated that cations are preferentially absorbed from a neutral salt solution; the effect was found to be very pH dependant.

The effectiveness of each particular phosphate should be mentioned. Hamm (1960) reported the following series for the hydration effect. In increasing order:

sodium monophosphate	(orthophosphate)
sodium cyclotriphosphate	(metaphosphate)
sodium diphosphate	(pyrophosphate)
sodium tetraphosphate	(polyphosphate)
sodium triphosphate	(polyphosphate)

(For an account of the nomenclature used see the appendix.)

Kormendy (1956) reported that straight chain polyphosphates were more efficient in increasing the water binding capacity of meat than pyro- and metaphosphates. Hellendorn (1962) stated that pyrophosphate and tripolyphosphate were most effective. Orthophosphate, trimetaphosphate and tetrametaphosphate showed little effect. Very recently Miller and Harrison (1965) showed that marination with sodium hexametaphosphate caused no significant change in the water holding capacity of muscle.

This review of literature which has been undertaken shows great inconsistency as to the merits of the various phosphates in increasing hydration of meat. Several reasons for this discrepancy can be indicated:

i. There is a great difference in the methods of study adopted by the various authors.

ii. The phosphates are investigated with and without added salts, and in various concentrations. Since their activity is affected by slight changes in conditions, small variations may cause appreciable differences in results.

3. Addition of Citrates

Very little work has been reported in the literature regarding the effect of added citrate on meat quality.

Ono et al. (1958) reported that the addition of citrate increased the water binding of boiled sausage. Hamm (1960) confirmed

this statement reporting that citrate had an effect similar to that of phosphate. It removed the alkaline earth metals bound to the meat protein by forming complexes with them. A solution containing three parts sodium citrate to one part sodium chloride was used by Benk (1952). The effect of this citrate admixture upon meat was shown to be unlike that caused by phosphate.

Wierbicki (1957b) stated that citric acid increased shrinkage when added to meat prior to heating, this having an adverse effect on meat quality. Hellendorn (1962) using citric acid in the form of its crystalline trisodium salt, $Na_3C_6H_5O_7.2H_2O$, concluded that citric acid does not exert any specific activity.

EXPERIMENTAL SECTION

A. BEEF UTILIZED IN THE INVESTIGATION

1. Experimentally controlled factors

One hip of beef, cut in approximately 3/4 inch steaks, was used in the current investigation. The meat came from an 18 month old Hereford steer that had been finished in a feed lot for four months prior to slaughter. During this feeding period the animal received one to two pounds of hay and 15 to 20 pounds grain plus supplements per day. No feed (water only) was allowed during the last 30 hours before slaughter.

The steer was killed on November 18, 1965. From the time of death to December 1, the carcass was aged in a storage room where the temperature was maintained at 38°F (3°C) with a relative humidity of 90%. On December 1, 1965 the carcass was cut up.

The hip section was sliced into 3/4 inch steaks which were put on trays and blast frozen at -30°F (-34°C). Twenty-four hours later the steaks were packed into two cartons and held in a storage freezer at -10°F (-23°C). On December 6 the meat was transferred to a second freezer at -5°F (-20°C) and kept there until the samples were removed for analysis.

2. Methods of sampling

Each of the steaks used in the investigation was identified by a number which indicated the location in the hip of the steer. The numbers started with 1 closest to the butt end and increasing towards the shank end. The arrangement is shown in Figure 1. Each of the steak sections was wrapped individually with the sample number marked clearly on the outside. Samples for analysis were selected at random.

Figure 2 illustrates the various cuts obtained from the hip.

Figure 3 shows a schematic diagram of a typical sample used in the investigation. In several cases the meat sample was sufficiently large for two series of analyses to be performed. In such instances the sample was cut in half with subsection (a) including the round bone. Analyses were then performed on each section.

B. PREPARATION OF SAMPLES

1. Preparation of untreated meat samples

A meat sample was taken from the freezer and placed in a cold storage room at 4°C for twenty-four hours to allow for complete thawing. Rigid time factors were maintained to minimize errors which may result from variations. After the meat sample had thawed, it was cut into 3/4 inch cubes. Meat samples were

Fig. 1. - Beef hip indicating sample numbers.

The beef was cut in approximately 3/4" steaks with number 1 being at the butt end and sample numbers increasing towards the shank end.

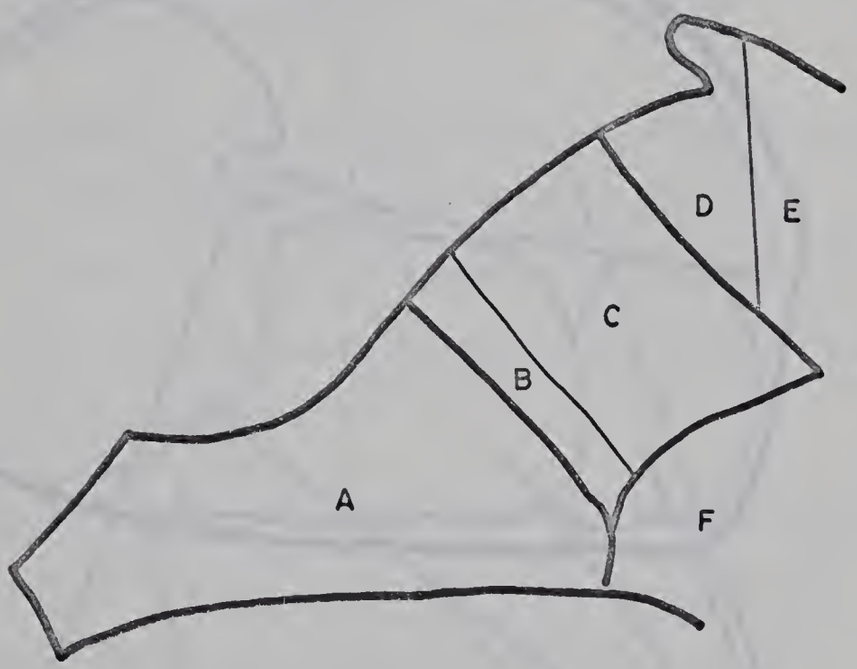


Fig. 2. - Beef hip indicating location of various cuts.

A. HIND SHANK

B. HEEL OF ROUND

C. ROUND

D. ROUND RUMP

E. SQUARE END RUMP

F. SIRLOIN TIP

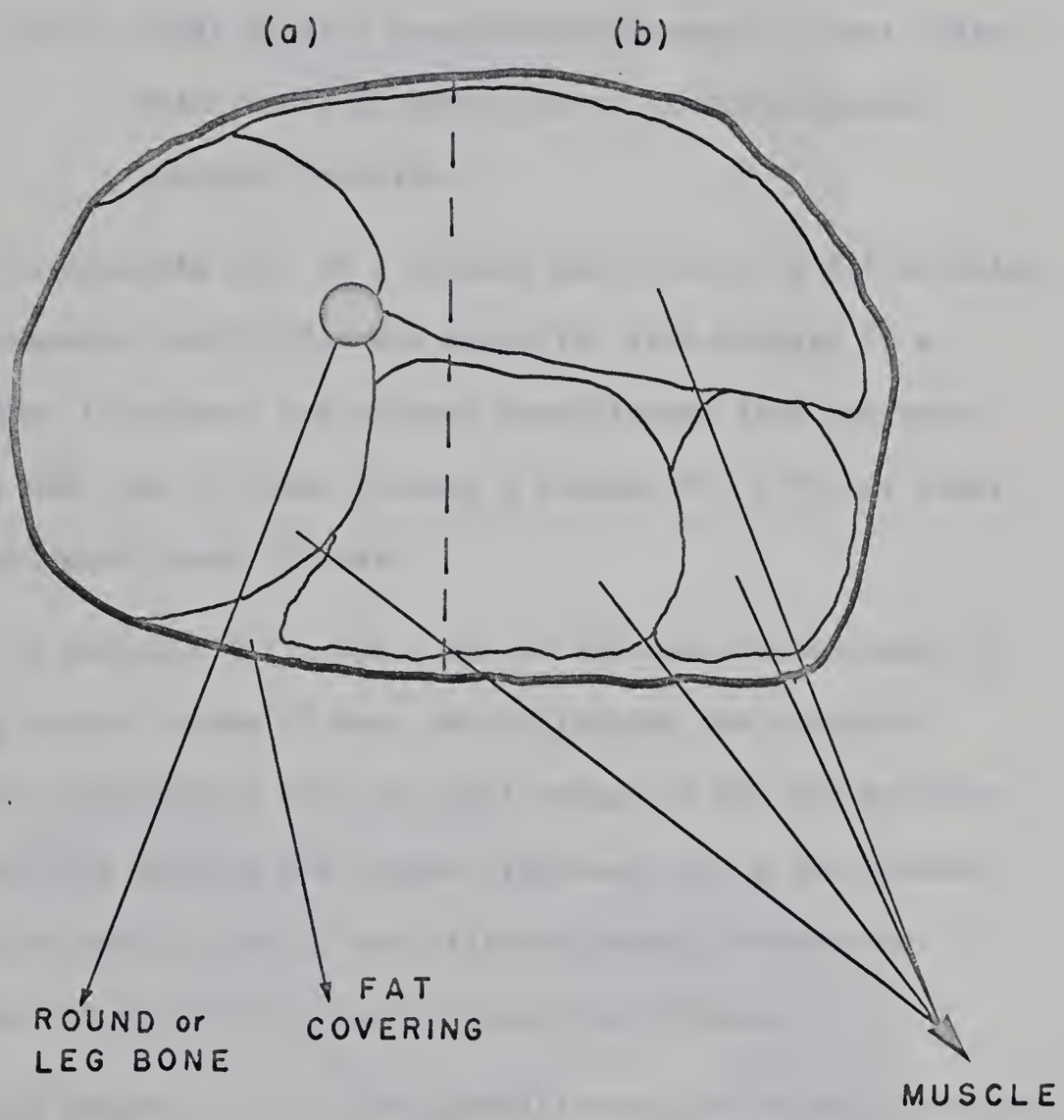


Fig. 3 - Schematic diagram of typical sample.

subjected to three types of analysis:

(i) total mineral constituents present,

(ii) total mineral constituents present in the juice extruded from meat, and

(iii) total mineral constituents present in meat juice which had been subjected to ultrafiltration (soluble fraction).

In analysis (i), 20 g of meat and 20 ml of a 20% solution of trichloroacetic acid (TCA) were mixed for five minutes in a Waring Blendor to extract the mineral constituents from the meat. The mixture was then filtered through a Whatman No. 1 filter paper giving a yellowish clear filtrate.

In analysis (ii), the juice of the meat was obtained by compressing several cubes of meat and collecting the extruded liquid. This was treated with an equal volume of 20% TCA solution and the resulting mixture was shaken vigorously for a few minutes. It was then allowed to settle and filtered through Whatman no. 1 filter paper. A yellowish clear filtrate was obtained.

In analysis (iii), the ultrafiltrate, or soluble fraction, of meat was obtained by filtering a portion of the juice obtained in the first step of analysis (ii) through a cellophane membrane using an ultrafilter apparatus similar to that of Ambard & Trautman (1960). This apparatus is shown in Figures 4 and 5. The juice is

Fig. 4. Ultrafilter used for the separation of the soluble fraction in meat.

Fig. 5. Ultrafilter disassembled.

forced through the membrane by the application of nitrogen gas under pressure. Fifty ml of the meat juice were subjected to ultrafiltration under a pressure of 16 - 20 lb/sq.in. at a temperature of 4°C. About 20 ml of ultrafiltrate were collected in the course of 18 hours.

2. Preparation of phosphate- or citrate-treated meat samples

The preparation of these samples was similar to that of those in section 1 above. During the twenty-fourth hour of thawing the meat was salted with one of a number of citrate or phosphate compounds. The following salt additives were used: monobasic sodium phosphate, dibasic sodium phosphate, sodium pyrophosphate, sodium tripolyphosphate, sodium tetraphosphate, sodium hexametaphosphate and sodium citrate. These salts were allowed to penetrate into the meat for one hour before the samples were cut into cubes.

The preparation of each of the fractions from the citrate-treated meat was identical to that for the untreated meat samples. However, in the case of phosphate-treated meat, the samples for analysis (i) were hydrolyzed using the method of Odagiri and Nickerson (1964). Twenty g of meat in 20 ml of 20% TCA solution were refluxed at a pH of 1.1 for 4 hours, converting all the phosphates present to the ortho form. This allowed for a measurement of both the inorganic phosphorus present in the meat and that which had been added.

3. Hydrolysis of phosphates in meat

Twenty g of meat was weighed into a beaker containing either 20 ml of water or TCA depending on the specific fraction desired. Enough of each phosphate was also added to increase the total phosphorus content by 50 mg per 100 g of meat. A blank containing no added phosphate was run. The samples were ground in the Waring Blendor and hydrolyzed using the method of Odagiri and Nickerson (1964). After the samples were refluxed for 4 hours at a pH of less than 2.5 they were filtered through Whatman No. 1 paper and the filtrates were set aside for chemical analysis.

C. CHEMICAL METHODS OF ANALYSIS

1. Determination of calcium

(a) Total calcium: The total calcium was determined in analyses (i) and (ii) by the improved complexometric method of Abd-el-Raheem (1957). This method was adapted for use in meat analysis with the following modifications:

1. Deionized water was used,
2. 0.005N EDTA was prepared from B.D.H. concentrated solutions,
3. The indicator used was Patton and Reeder's reagent: 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid. (Patton and Reeder, 1956). One gram of this reagent was finely ground with 100 g

of sodium chloride. This indicator was used in place of Erio S.E.,

4. Hydroxylamine hydrochloride was used in place of aluminum nitrate. It was found that the former was much more efficient in masking phosphate interference.

The meat preparation was diluted 10 times with water and 20 ml of this solution were transferred into a titration flask. Reagents were added in the following specific order:

(a) a few milligrams of hydroxylamine hydrochloride,

(b) a small crystal of potassium cyanide,

(c) 6 - 7 drops of 50% sodium hydroxide solution,

(d) a few milligrams of indicator-salt mixture.

The solution was then titrated with the standard EDTA solution. A tungsten light source was used to distinguish the end-point more easily.

(b) Soluble calcium: the quantity of calcium present in the ultrafiltrate solution was determined in the same manner as the total calcium in (a) above. However an initial dilution factor of 20 was used, 20 ml of the resulting solution being titrated.

2. Determination of magnesium

(a) Total magnesium: the total magnesium present in analyses (i) and (ii) above was determined by the method of Gjems (1960) with the following modifications:

1. Deionized water was used,
2. 1 g of Eriochrome Black T was finely ground with 100 g of sodium chloride.

The meat preparation was diluted 10 times with water; 20 ml of this solution were transferred to a titration flask and reagents were added in the following order:

(a) 5 ml of 10% hydroxylamine hydrochloride solution,

(b) 5 ml of ammonia/ammonium chloride buffer - pH 10; 13.5 g Ammonium chloride and 88 ml concentrated ammonia was diluted to 250 ml with deionized water,

(c) a small crystal of potassium cyanide,

(d) a few milligrams of Eriochrome Black T-salt mixture.

The solution was titrated with EDTA solution; a tungsten light source being used for easier detection of the end-point.

(b) Soluble Magnesium: The quantity of soluble magnesium was determined in the same manner as was used for total magnesium. However, an initial dilution factor of 20 was used, 20 ml of the resulting solution being titrated.

It should be mentioned here that the above method determines the quantities of both calcium and magnesium present, thus the content of magnesium is determined by difference.

3. Determination of citric acid

Total and soluble citric acid were determined in the TCA filtrate and ultrafiltrate respectively by the improved pyridine/acetic anhydride method of Marier and Boulet (1958). This method was developed originally for the determination of citric acid in milk, however, no difficulties were encountered when the method was applied to meat filtrates.

No dilution of the filtrates was necessary, with the exception of the meat samples to which citrate had been added. In this instance the filtrates were diluted so that the citric acid content was between 50 and 200 mg/ml. A blank which took into account the original color of the ultrafiltrate solution was necessary. In this blank glacial acetic acid was added in the place of acetic anhydride. The blank gave no color reaction.

The calibration curve obtained with anhydrous citric acid is shown in Figure 6.

4. Determination of inorganic phosphate

The quantities of inorganic phosphate were determined by the method of Polley (1949).

Samples of the TCA filtrate and ultrafiltrate were diluted in such a manner as to have a phosphorus content within the range of the calibration curve, i.e. between 0.2 and 5.0 mg of KH_2PO_4/100 ml. The calibration curve obtained with monopotassium phosphate standards is shown in Figure 7. The concentration of inorganic phosphate was expressed in terms of actual phosphorus.

D. PHYSICAL METHODS OF ANALYSIS

1. Determination of moisture

(a) Total moisture: the total moisture in meat was determined by the method (23.002) of the Association of Official Agricultural Chemists (1965). Moisture was reported as loss in weight of the meat sample.

(b) Free moisture: the free moisture in meat was determined by the method of Wierbicki and Deatherage (1958). This involved pressing a freshly-cut 400 - 600 mg sample on filter paper under a constant pressure of 500 p.s.i. for 1 minute. The constant pressure was obtained with a Wabash Laboratory Press.

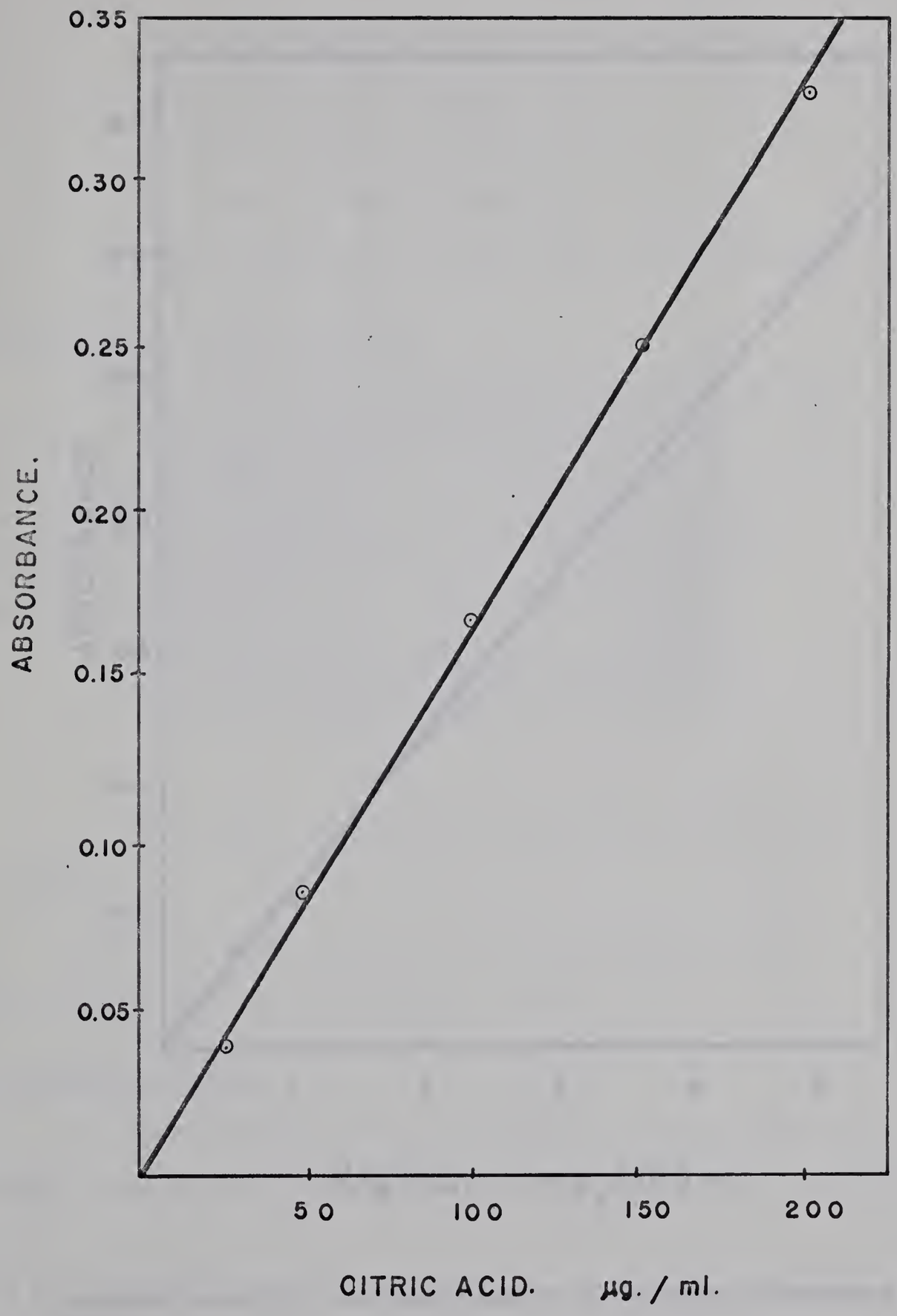
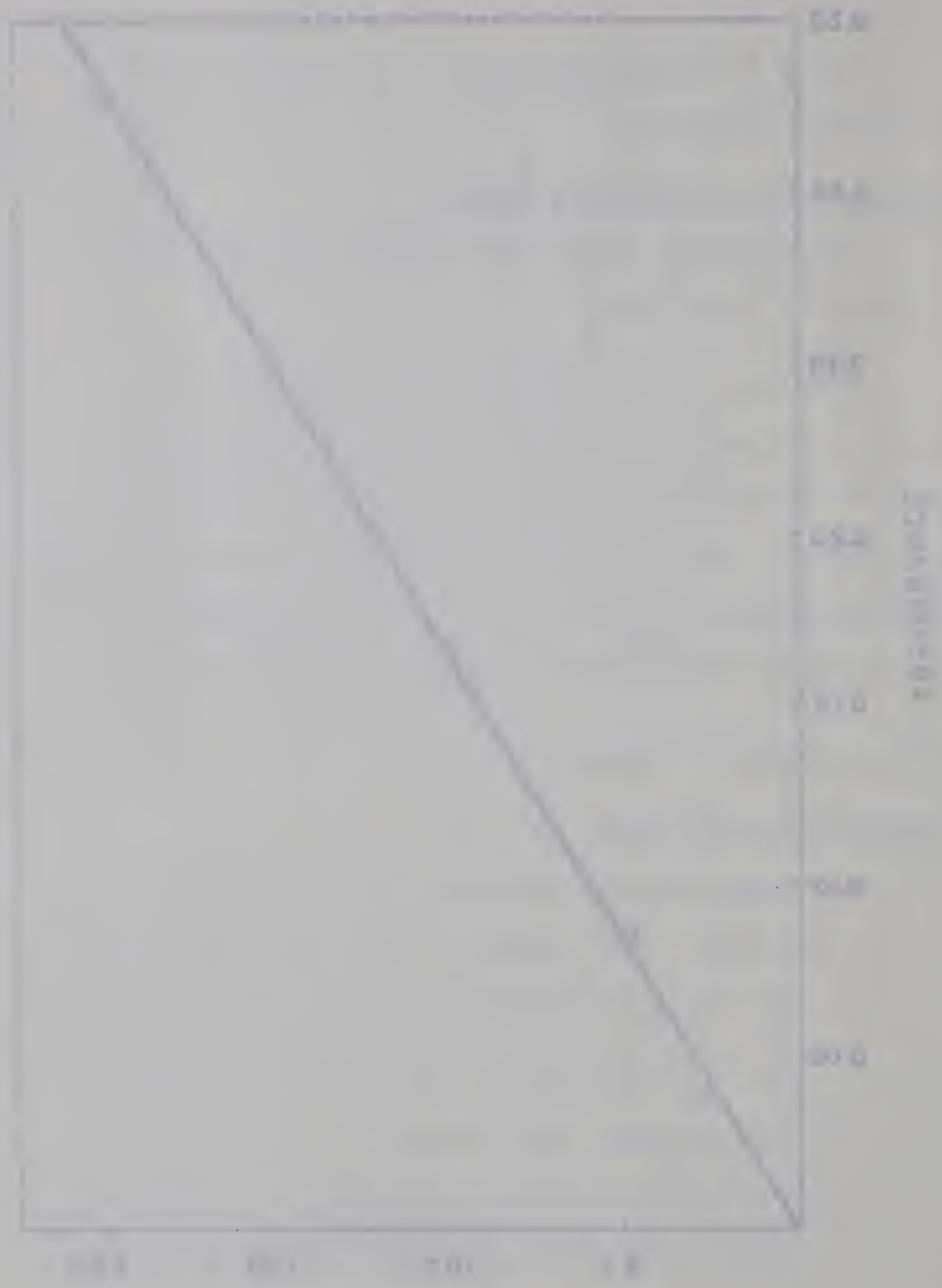


Fig. 6 - Standard curve for the determination of citric acid in meat.



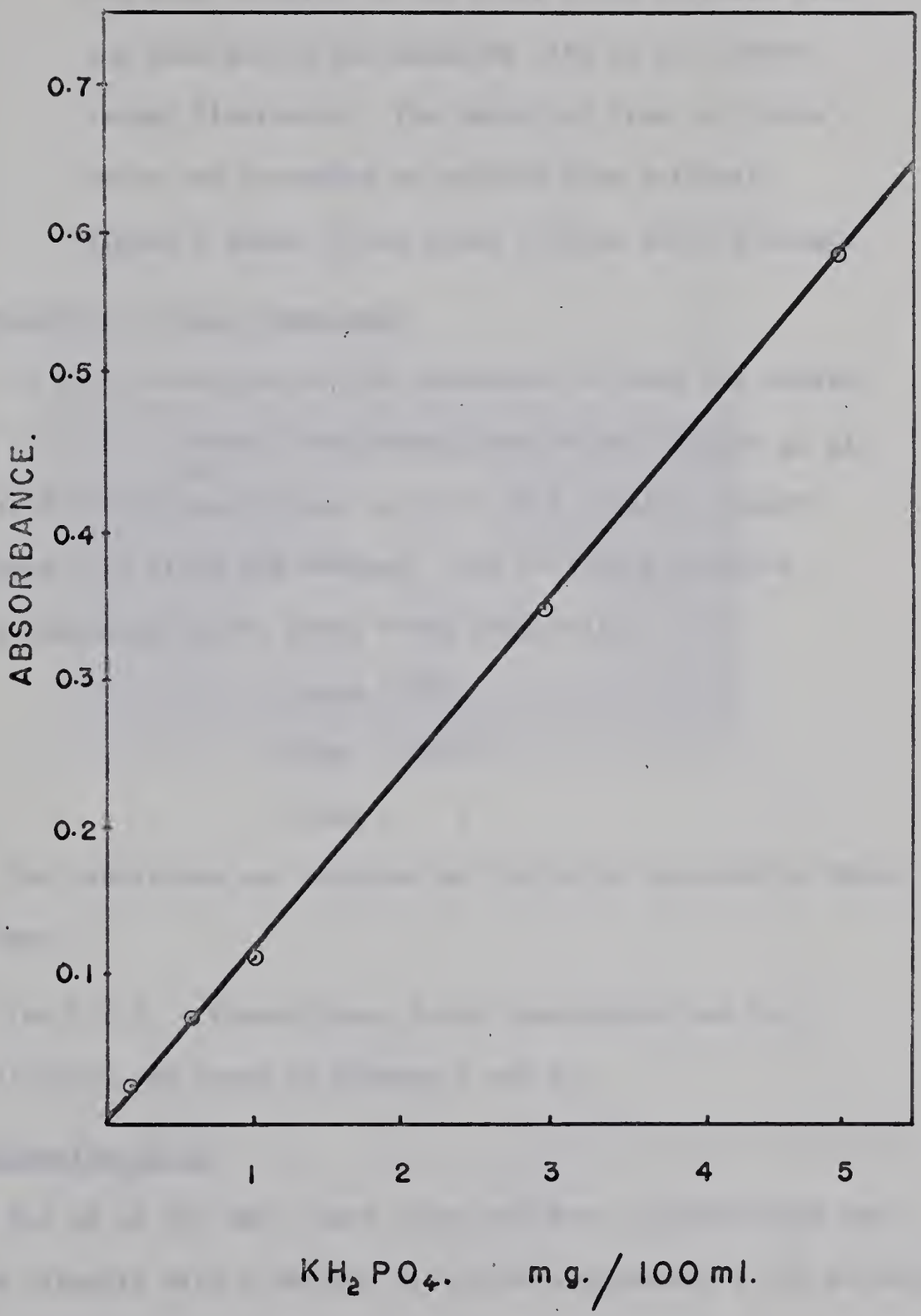


Fig. 7 - Standard curve for the determination of inorganic phosphorus in meat.

The area occupied by the water which diffused from the meat sample was measured with an Ott Compensating Planimeter. The amount of free or "loose" water was expressed as percent free moisture. Figure 8 shows filter paper circles after pressing.

2. Determination of meat tenderness

In this investigation the tenderness of meat was determined with a L.E.E. - Kramer Shear Press Comptroller (Bailey et al., 1962). The 3/4 inch steaks were cut into 10 g samples, placed in the Kramer cell block and sheared. The following were the standard adjustments on the Shear Press Comptroller

Range - 1000

Ring - 3000

Speed - 1

The tenderness was reported as the force required to shear 100 g of meat.

The L.E.E. - Kramer Shear Press Comptroller and the sample cell block are shown in Figures 9 and 10.

3. Determination of pH

The pH of the meat, meat juice and meat ultrafiltrate was determined directly with a Metrohm Precision Compensator E 222 pH Meter.

Fig. 8. Free moisture in meat. Filter paper circles after pressing.

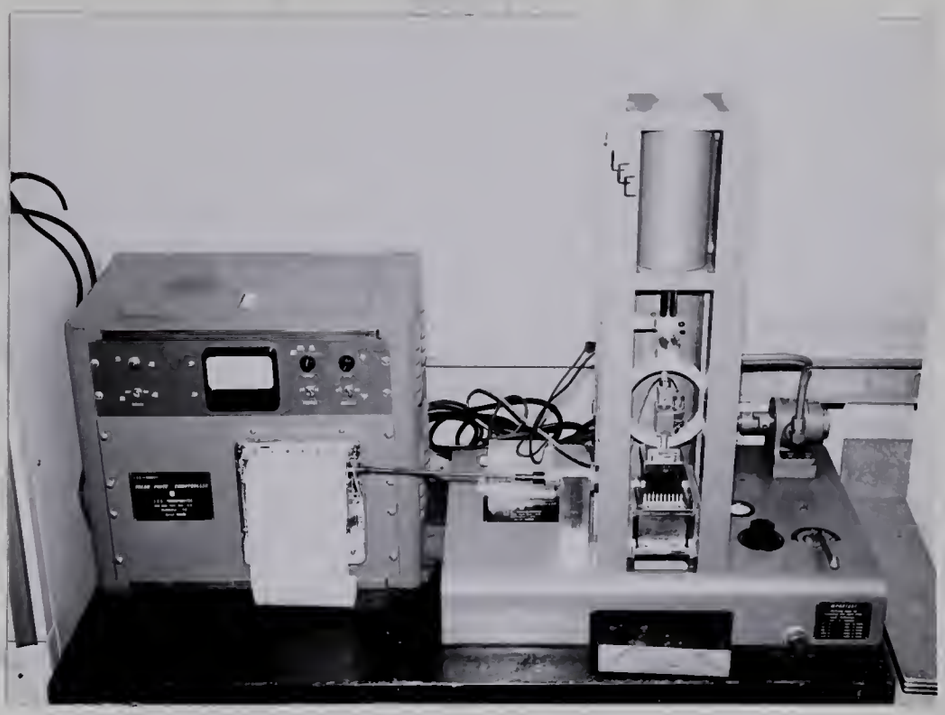


Fig. 9. L.E.E. - Kramer Shear Press Comptroller used in the determination of tenderness of meat.

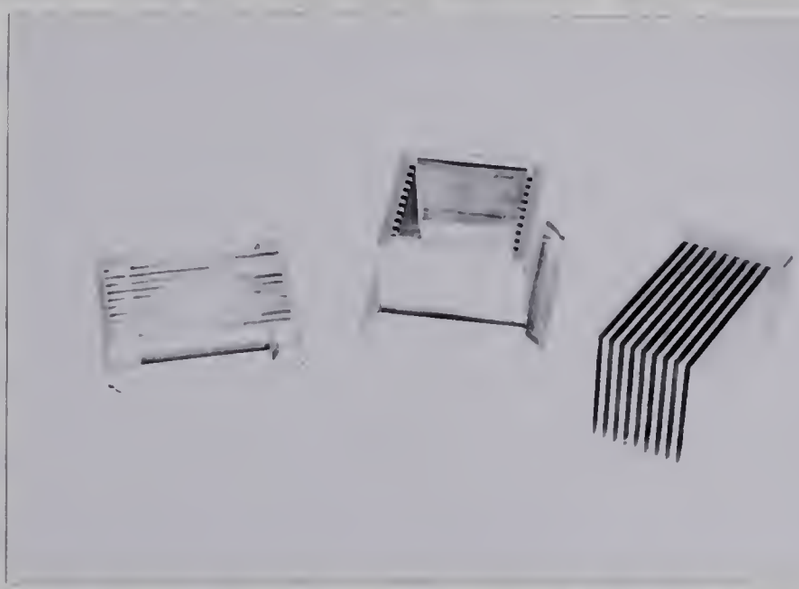


Fig. 10. Shear cell.

RESULTS

A. INVESTIGATION OF MEAT SAMPLES FOR TENDERNESS VALUES, pH, MOISTURE RELATIONSHIPS, CALCIUM AND MAGNESIUM CONTENT, AND CITRIC ACID AND PHOSPHORUS CONTENT.

1. Untreated meat

The tenderness of the untreated meat is shown in Table 2. Values ranged from 807.6 lbs/100g to 987.9 lbs/100g meat.

The pH of the meat, the meat juice and the meat ultrafiltrate was determined and results reported in Table 3. No pH differences were observed between the three products.

The total and free moisture content of the meat is reported in Table 4. The total moisture content did not vary much from sample to sample. The mean was 73.47% and the standard deviation 0.98%. However, the free moisture determination is subject to a greater experimental error, and the standard deviation was 5.99%.

The results of total and soluble calcium and magnesium determinations are reported in Tables 5 and 6 respectively. A mean of 8.61 mg total calcium/100g, and 3.83 mg soluble calcium/100g meat was found. Both the total and soluble calcium were higher in samples "a" which were located nearest the round bone. The mean value for total magnesium was 24.35 mg/100g and for soluble magnesium 17.68 mg/100g.

The total citric acid content of the meat was found to have a mean value of 8.16 mg/100g (Table 7). The soluble citric acid

content was only slightly less indicating that most of the citric acid was present in the free form. Similarly, the inorganic phosphorus of the meat occurred primarily in the soluble form (Table 8). The mean soluble phosphorus content was 95.21 mg/100g. The mean total phosphorus content was 116.42 mg/100g.

2. Citrate-treated meat

Sodium citrate was salted on the meat and changes resulting from this treatment were determined. The amount of citrate added was in the range of 220 - 450 mg/100g.

A mean tenderness value of 876.4 lbs/100g was obtained, the standard deviation was 153.6 lbs/100g (Table 9).

The mean values of 5.77, 5.75 and 5.80 are reported in Table 10 for the pH of the meat, the meat juice and the meat ultrafiltrate respectively.

The results in Table 11 show the total and free moisture content in citrate-treated meat. The mean total moisture was 73.14% and the mean free moisture content was 54.77%.

The calcium and magnesium content of the citrate treated meat is shown in Tables 12 and 13 respectively. 9.31 mg/100g is reported as the mean total calcium and 5.36 is reported as the mean soluble calcium. Samples "a" which are located nearest the round bone had a higher calcium content than samples "b". The mean value for total magnesium was 24.74 mg/100g and soluble magnesium

was 17.86 mg/100g meat.

Table 14 gives the inorganic phosphorus content of the citrate-treated meat. Mean total inorganic phosphorus content was found to be 118.24 mg/100g and the mean soluble inorganic phosphorus content was 101.47 mg/100g.

The distribution of added citrate in the meat is given in Table 15. Approximately two-thirds of the citric acid measured was in the soluble form.

3. Phosphate-treated meat

Table 16 lists the total and soluble inorganic phosphorus content of meat samples which had been subjected to different phosphate treatments. Other variables for these samples are reported in Table 17. Each different phosphate exerts a specific effect; therefore, the results are not discussed as a whole at this point. Each is reported individually.

Tenderness appeared to increase somewhat in the meat samples which had been salted with dibasic sodium phosphate, sodium tripolyphosphate, sodium tetraphosphate and sodium hexametaphosphate. The samples which were treated with sodium phosphate (dibasic), sodium pyrophosphate, sodium tripolyphosphate and sodium hexametaphosphate showed an increase in pH as compared with the samples treated with sodium phosphate (monobasic) and sodium tetraphosphate. Addition of dibasic sodium phosphate resulted in an increase of approximately 0.40 units, the greatest change observed. The meat

samples treated with phosphates showed no change in free moisture or water holding capacity.

B. COMPARISONS OF TENDERNESS VALUES, pH, MOISTURE RELATIONSHIP, CALCIUM AND MAGNESIUM CONTENT, AND CITRIC ACID AND PHOSPHORUS CONTENT OF THE UNTREATED, CITRATE-TREATED, AND THE VARIOUS PHOSPHATE-TREATED MEAT SAMPLES

Results of determinations made on untreated, citrate-treated and phosphate-treated samples are shown in Table 18. Analysis of variance was carried out to determine whether the affects of the various treatments on the tenderness of meat were significantly different. F was significant at the 5% level. Analysis using Duncan's Multiple Range test showed that the tenderness of phosphate-treated meat was statistically different from that of the control. Tenderness of the citrate-treated meat was not significantly different from the control.

The pH values of meat, meat juice and meat ultrafiltrate were significantly different after citrate and phosphate treatment. Analysis with Duncan's Multiple Range test showed that the pH values of phosphate-treated samples were significantly different from the untreated; pH values of citrate-treated samples were also significantly different.

The total and free moisture content remained unchanged. Concentrations of total calcium and soluble calcium were not

sifnificantly different after citrate and phosphate treatment. The difference in the mean magnesium, citric acid and inorganic phosphorus content was also not significantly different.

C. THE RELATIVE DEGREE OF BINDING OF CALCIUM AND MAGNESIUM, CITRIC ACID AND PHOSPHORUS IN MEAT TREATED WITH CITRATE AND PHOSPHATES.

The distribution of calcium in meat samples treated with citrate and phosphates is given in Table 19. Analyses are reported for the calcium bound to insoluble protein. The calcium bound to soluble protein and the colloidal calcium was also determined and was recorded as one value. The soluble protein-bound calcium and colloidal calcium increased as the chain length of the phosphate additive increased. However, the amount of calcium bound to insoluble protein decreased as the chain length increased. One can therefore postulate that added phosphate removes calcium from its bound state with the insoluble protein. Sodium citrate appeared to have the same effect although to a lesser extent.

Magnesium concentrations were shown to be unaffected by the presence of added salts as indicated in Table 20.

No specific trends are noted in the distribution of citric acid and inorganic phosphorus which are shown in Table 21 and 22 respectively. However, most of the added citrate and phosphate was bound to the insoluble protein rather than bound to the soluble protein or in the colloidal form.

Table 23 presents results on the water-extractable inorganic phosphorus, and acid-extractable inorganic phosphorus both in the unhydrolyzed and hydrolyzed form. Difficulties were encountered in the determination of phosphorus in samples treated with polyphosphates. The nature of the test was such that phosphorus in the form of orthophosphate could only be determined. It was shown that hydrolysis is necessary to change the polyphosphates to the ortho form for the determination of the total phosphorus content.

Table 2. Tenderness of untreated meat samples with mean and standard deviation. (Tenderness is expressed as the pounds of force required to shear 100g meat.)

Sample Number	Tenderness (lbs/100g meat)
14b	971.7
5a	926.7
3b	852.7
12a	838.2
4a	807.6
13b	987.9
Mean	897.5
Standard deviation	75.0

Table 3. pH of meat, juice and ultrafiltrate of untreated meat samples with mean and standard deviation.

Sample Number	pH meat	pH juice	pH ultrafiltrate
14b	5.47	5.47	5.57
5a	5.62	5.62	5.60
3b	5.63	5.63	5.64
12a	5.66	5.66	5.65
4a	5.60	5.61	5.70
13b	5.56	5.64	5.58
Mean	5.59	5.60	5.62
Standard deviation	0.04	0.06	0.04

Table 4. Total moisture and free moisture content of untreated meat samples with mean and standard deviation.

Sample Number	Total moisture (%)	Free moisture (%)
14b	73.66	49.89
5a	72.90	45.04
3b	73.23	56.80
12a	75.14	57.80
4a	73.67	49.25
13b	72.22	60.42
Mean	73.47	53.20
Standard deviation	0.98	5.99

Table 5. Total and soluble calcium content of untreated meat samples with mean and standard deviation.

Sample number	Total calcium (mg/100g meat)	Soluble calcium (mg/100g meat)
14b	3.91	1.92
5a	9.96	4.09
3b	3.82	2.20
12a	14.11	5.57
4a	9.29	5.61
13b	10.57	3.62
Mean	8.61	3.83
Standard deviation	4.03	2.12

Table 6. Total and soluble magnesium content of untreated meat samples with mean and standard deviation.

Sample number	Total magnesium (mg/100g meat)	Soluble magnesium (mg/100g meat)
14b	30.30	21.94
5a	23.06	17.73
3b	22.41	16.24
12a	24.71	18.36
4a	21.01	18.49
13b	24.64	13.33
Mean	24.35	17.68
Standard deviation	3.23	2.84

Table 7. Total and soluble citric acid content of untreated meat samples with mean and standard deviation.

Sample number	Total citric acid (mg/100g meat)	Soluble citric acid (mg/100g meat)
14b	7.81	6.99
5a	10.37	7.14
3b	5.63	2.56
12a	10.01	9.77
4a	8.25	7.98
13b	6.89	4.87
Mean	8.16	6.55
Standard deviation	2.17	2.52

Table 8. Total and soluble inorganic phosphorus content of untreated meat samples with mean and standard deviation.

Sample number	Total inorganic phosphorus (mg/100g meat)	Soluble inorganic phosphorus (mg/100g meat)
14b	100.78	85.50
5a	116.09	94.57
3b	132.10	102.52
12a	121.58	102.61
4a	126.47	100.60
13b	101.90	85.47
Mean	116.49	95.21
Standard deviation	12.88	8.09

Table 9. Tenderness of citrate treated* meat samples with mean and standard deviation. (Tenderness is expressed as the pounds of force required to shear 100g meat.)

Sample number	Tenderness (lbs/100g meat)
5b	966.0
3a	737.7
17	1060.4
12b	924.7
11a	647.8
10b	921.7
Mean	876.4
Standard deviation	153.6

*Amount citrate added was in the range 220-450 mg/100g meat.

Table 10. pH of meat, juice and ultrafiltrate of citrate treated* meat samples with mean and standard deviation.

Sample number	pH meat	pH juice	pH ultrafiltrate
5b	5.67	5.70	5.77
3a	5.81	5.75	5.81
17	5.81	5.77	5.84
12b	5.74	5.74	5.80
11a	5.86	5.78	5.78
10b	5.73	5.74	5.79
Mean	5.77	5.75	5.80
Standard deviation	0.06	0.04	0.04

*Amount citrate added was in the range 220-450 mg/100g meat.

Table 11. Total moisture and free moisture content of citrate treated* meat samples with mean and standard deviation.

Sample number	Total moisture (%)	Free moisture (%)
5b	73.59	52.52
3a	73.14	52.92
17	73.54	49.30
12b	72.49	62.67
11a	72.88	56.08
10b	73.18	55.16
Mean	73.14	54.77
Standard deviation	0.38	4.53

*Amount citrate added was in the range 220-450 mg/100g meat.

Table 12. Total and soluble calcium content of citrate treated* meat samples with mean and standard deviation.

Sample number	Total calcium (mg/100g meat)	Soluble calcium (mg/100g meat)
5b	5.31	3.24
3a	13.77	5.24
17	4.05	2.80
12b	10.05	8.40
11a	13.17	8.18
10b	9.51	4.31
Mean	9.31	5.36
Standard deviation	3.95	2.42

*Amount citrate added was in the range 220-450 mg/100g meat.

Table 13. Total and soluble magnesium content of citrate treated* meat samples with mean and standard deviation.

Sample number	Total magnesium (mg/100g meat)	Soluble magnesium (mg/100g meat)
5b	27.55	20.13
3a	25.37	18.93
17	26.69	21.14
12b	27.58	19.83
11a	22.40	14.00
10b	18.82	13.12
Mean	24.74	17.86
Standard deviation	3.50	3.42

*Amount citrate added was in the range 220-450 mg/100g meat.

Table 14. Total and soluble inorganic phosphorus content of citrate treated* meat samples with mean and standard deviation.

Sample number	Total inorganic phosphorus (mg/100g meat)	Soluble inorganic phosphorus (mg/100g meat)
5b	134.34	122.27
3a	106.40	104.22
17	114.55	102.10
12b	117.78	90.74
11a	118.03	96.21
10b	110.35	93.27
Mean	118.24	101.47
Standard deviation	9.08	11.40

*Amount citrate added was in the range 220-450 mg/100g meat.

Table 15. Total and soluble citric acid content of citrate treated meat. The amount of citrate added is given.

Sample number	Sodium citrate added (mg/100g meat)	Citrate added (mg/100g meat)	Total citric acid	Soluble citric acid
5b	341.2	219.22	294.4	194.4
3a	710.4	456.43	451.9	309.3
17	560.0	359.80	378.0	260.9
12b	479.0	307.76	380.6	296.2
11a	379.7	243.96	270.1	209.5
10b	530.2	340.65	398.3	256.1

Table 16. Total and soluble inorganic phosphorus content of meat receiving different phosphate treatments. The amount of phosphorus in each treatment is given.

Sample number	Phosphate salted	Amount added (mg/100g meat)	Amount of actual effective phosphorus (mg/100g meat)	Total inorganic phosphorus (mg/100g meat)*	Soluble inorganic phosphorus (mg/100g meat)
2	Monobasic sodium phosphate	526.9	118.08	214.92	212.09
7b	Dibasic sodium phosphate	453.5	94.98	224.29	146.22
13a	Sodium pyrophosphate	410.7	52.94	196.51	115.84
7a	Sodium tripolyphosphate	393.5	99.36	167.65	103.52
11b	Sodium tetraphosphate	364.6	96.11	156.09	98.36
10a	Sodium hexametaphosphate	494.3	150.12	178.60	99.16

*Determined after hydrolysis.

Table 17. The values for various determinations for meat receiving different phosphate treatments.

Variable	Monobasic sodium phosphate	Dibasic sodium phosphate	Sodium pyro-phosphate	Sodium tripoly-phosphate	Sodium tetra-phosphate	Sodium hexameta-phosphate
Tenderness (lbs/100g meat)	935.3	653.1	820.6	623.1	685.2	643.5
pH meat	5.57	5.98	5.82	5.82	5.54	5.84
pH juice	5.58	6.05	5.84	5.81	5.58	5.76
pH ultrafiltrate	5.58	5.99	5.84	5.81	5.57	5.69
Total moisture (%)	71.68	71.38	72.71	73.33	72.03	72.61
Free moisture (%)	52.12	55.18	55.07	53.65	55.13	62.41
Total calcium (mg/100g meat)	10.02	10.04	8.95	15.63	9.47	9.46
Soluble calcium (mg/100g meat)	4.94	4.29	4.28	8.58	4.18	4.36
Total magnesium (mg/100g meat)	23.16	18.99	19.67	23.48	20.41	24.46
Soluble magnesium (mg/100g meat)	16.85	15.01	13.30	16.78	9.55	16.15
Total citric acid (mg/100g meat)	9.44	11.14	4.32	9.01	7.75	12.94
Soluble citric acid (mg/100g meat)	7.53	10.71	3.01	8.07	5.40	7.62
Sample number	2	7b	13a	7a	11b	10a

Table 18. The mean values for various determinations for untreated, citrate-treated and phosphate-treated meat (A comparison)

Variable	Untreated meat	Citrate-treated meat	Phosphate-treated meat
Tenderness (lbs/100g meat)	897.5	876.4	726.8
pH meat	5.59	5.77	5.76
pH juice	5.60	5.75	5.77
pH ultrafiltrate	5.62	5.80	5.75
Total moisture (%)	73.47	73.14	72.29
Free moisture (%)	53.20	54.77	55.59
Total calcium (mg/100g meat)	8.61	9.31	10.59
Soluble calcium (mg/100g meat)	3.83	5.36	5.10
Total magnesium (mg/100g meat)	24.35	24.74	21.69
Soluble magnesium (mg/100g meat)	17.68	17.86	14.62
Total citric acid (mg/100g meat)	8.16	-	9.10
Soluble citric acid (mg/100g meat)	6.55	-	7.06
Total inorganic phosphorus (mg/100g meat)	116.49	118.24	-
Soluble inorganic phosphorus (mg/100g meat)	95.21	101.47	-

Table 19. Distribution of calcium in meat receiving various chemical treatments.*

Variable	Untreated meat	Citrate-treated meat	Phosphate-treated meat					
		Sodium citrate	Monobasic sodium phosphate	Dibasic sodium phosphate	Sodium pyro-phosphate	Sodium tripoly-phosphate	Sodium tetra-phosphate	Sodium hexameta-phosphate
Total calcium in meat	10.57	13.17	11.34	10.56	9.46	15.63	9.16	10.80
Calcium bound to insoluble protein	5.07	2.54	4.04	4.72	2.18	2.26	0.76	0.21
Calcium bound to soluble protein and colloidal calcium	1.88	2.45	2.36	1.55	3.00	4.79	4.32	6.23
Soluble calcium	3.62	8.18	4.94	4.29	4.28	8.58	4.18	4.36
Total calcium in meat juice	5.50	10.63	7.30	5.84	7.28	13.37	8.40	10.59

*Results are expressed as mg calcium/100g meat.

Table 20. Distribution of magnesium in meat receiving various chemical treatments.*

Variable	Untreated meat	Citrate-treated meat: Sodium citrate	Phosphate-treated meat: Monobasic sodium phosphate	Dibasic sodium phosphate	Sodium pyro-phosphate	Sodium tripoly-phosphate	Sodium tetra-phosphate	Sodium hexameta-phosphate
Total magnesium in meat	24.64	22.40	13.47	18.99	19.67	23.48	20.41	15.04
Magnesium bound to insoluble protein	8.75	8.30	3.09	3.12	3.76	8.90	9.46	4.38
Magnesium bound to soluble protein and colloidal magnesium	2.56	0.07	-	0.86	2.61	1.80	1.40	0.51
Soluble magnesium	13.33	14.00	10.85	15.01	13.30	12.78	9.55	10.15
Total magnesium in meat juice	15.89	14.07	10.38	15.87	15.91	14.58	10.95	10.66

*Results are expressed as mg magnesium/100g meat.

Table 21. Distribution of citric acid in meat receiving various chemical treatments.*

Variable	Untreated meat	Citrate-treated meat	Phosphate-treated meat					
		Sodium citrate	Monobasic sodium phosphate	Dibasic sodium phosphate	Sodium pyro-phosphate	Sodium tripoly-phosphate	Sodium tetra-phosphate	Sodium hexameta-phosphate
Total citric acid	6.89	270.09	9.44	10.28	4.32	9.01	7.75	14.82
Citric acid bound to insoluble protein	1.12	60.56	1.42	-	0.69	1.38	1.99	2.93
Citric acid bound to soluble protein and colloidal citric acid	0.90	0.03	0.49	0.07	0.62	0.44	0.36	3.27
Soluble citric acid	4.87	209.56	7.53	10.71	3.01	8.07	5.40	7.62
Total citric acid in meat juice	5.77	209.53	8.02	11.78	3.63	7.63	5.76	10.89
Amount of effective citrate added	-	243.96	-	-	-	-	-	-

*Results are expressed as mg citric acid/100g meat.

Table 22. Distribution of inorganic phosphorus in meat receiving various chemical treatments.*[1]

Variable	Untreated meat	Citrate-treated meat	Phosphate-treated meat					
		Sodium citrate	Monobasic sodium phosphate	Dibasic sodium phosphate	Sodium pyro-phosphate	Sodium tripoly-phosphate	Sodium tetra-phosphate	Sodium hexameta-phosphate
Total inorganic phosphorus	101.90	118.03	214.92	224.29	196.51	167.65	156.09	178.60
Inorganic phosphorus bound to insoluble protein	12.44	11.87	19.12	53.71	64.12	52.91	24.94	62.95
Inorganic phosphorus bound to soluble protein and colloidal inorganic phosphorus	4.07	9.95	-	24.36	16.55	11.22	32.79	16.49
Soluble inorganic phosphorus	85.47	96.21	212.09	146.22	115.84	103.52	98.36	99.16
Total inorganic phosphorus in meat juice	89.54	106.16	195.80	170.58	132.39	114.74	131.15	115.65
Amount of effective phosphorus added	-	-	118.08	94.98	52.94	99.36	96.11	150.12

*Determined after hydrolysis
1 Results are expressed as mg inorganic phosphorus/100g meat.

Table 23. Hydrolysis of phosphates in meat. Effect on the total measurable inorganic phosphorus. Water-extractable inorganic phosphorus content is also given.*[1]

Test	Phosphate-treated meat						Untreated meat
	Monobasic sodium phosphate	Dibasic sodium phosphate	Sodium pyro-phosphate	Sodium tripoly-phosphate	Sodium tetra-phosphate	Sodium hexameta-phosphate	
Water-extractable inorganic phosphorus	131.87	125.53	112.36	117.71	117.38	98.09	96.12
Total inorganic phosphorus (unhydrolyzed)	148.74	151.12	117.69	117.68	111.82	113.77	96.12
Total inorganic phosphorus (hydrolyzed)	145.95	145.14	147.68	141.24	133.38	133.38	88.28

* Results are expressed as mg inorganic phosphorus/100g meat.
1 The amount of each phosphate added increased the phosphorus content 50mg/100g meat.

DISCUSSION

The reason for undertaking this investigation was to obtain information that might relate mineral equilibria to some of the physical properties of meat. It was hoped that this could be rationalized in terms of changes in ionic balance. The project was also designed to ascertain the effect of added phosphates or citrate on the water and ion-binding properties of meat.

It has been shown that heredity, species, sex, age, grade, breeding and feeding practices affect the quality of meat (American Meat Institute Foundation (1960); Palmer (1963)). In this investigation the variability due to these factors was minimized by the use of samples from a single animal. The way in which meat is handled may also induce chemical changes in the product. Changes might also be expected as a result of freezing. However, Deatherage (1956), Wierbicke *et al*. (1957b), American Meat Institute Foundation (1960) and Hamm (1960) have shown that if meat is blast frozen, as it was in this investigation, no changes could be detected. In any case, every attempt was made to keep the variability due to animal, storage and handling at a minimum.

Variations between different samples from the same animal could not be eliminated in the present study. This must be recognized as a source of error but it does not necessarily invalidate the findings of this investigation.

Emphasis was placed on the development of suitable methods for the determination of the various constituents in meat. It is, therefore, of value to discuss the results obtained in this investigation in the light of those reported by other authors.

As stated earlier, tenderness of meat is one of the most important quality factors determining its consumer acceptibility. For this reason extensive studies have been carried out to develop methods of measuring tenderness and relating this to the eating quality of meat. Because the sensory method is generally time-consuming and often not practical, physical or mechanical methods have been widely used. However, the results obtained by such means must be related to sensory tenderness values. In this investigation tenderness was measured with a L.E.E. - Kramer Shear Press. Statistically significant correlation coefficients between L.E.E. - Kramer Shear force and sensory tenderness values of beef steaks were obtained by Bailey et al. (1962) and Sharrah et al. (1965).

The results of the study of the calcium content of meat reported here are in general agreement with those reported by Mittledorf and London (1952), Swift and Berman (1959) and American Meat Institute Foundation (1960). Although there were differences in technique, the values obtained are similar. A range of 2.6 - 11.0 mg calcium/100g meat has been reported in the literature for the content of calcium in beef. In this investigation the values found were in this range; an average value of 10.59 mg/100g was found.

The results for the magnesium content of meat are also in agreement with those reported in the literature. Toscani (1945) reported an average content of 24.9 mg/100g with a range of 19.8 - 31.6 mg/100g. Middledorf and London (1952) reported a value of 25 mg magnesium/100g. An average value of 21.1 mg/100g with a range of 14.0 - 25.7 mg/100g was reported by Swift and Berman (1959) for a number of different samples. Fox et al. (1960) stated that the content of magnesium in beef is between 13.3 - 25.2 mg/100g with a mean value of 20.3 mg/100g. An overall average of 23.59 mg/100g was obtained in this study. The above figures represent the total content of calcium and magnesium in the samples used. Values for the content of these minerals present in the several forms reported in this thesis have not been previously reported.

Values for the inorganic phosphorus content of meat have been reported by Swift and Berman (1959) who found a range of 109 - 213 mg/100g. The content of citric acid in meat had not been previously reported in the literature.

Comparisons of determinations carried out on untreated, citrate-treated and phosphate-treated samples were made in an attempt to indicate the effect of each specific additive. The addition of dibasic sodium phosphate, sodium tripolyphosphate, sodium tetraphosphate and sodium hexametaphosphate caused the meat to become more tender. This effect is not necessarily an effect of pH because changes in pH do not account for changes in

tenderness. For example, the pH of sodium tetraphosphate treated meat does not vary from the untreated meat; however the tenderness is improved. Inversely, a change of 0.3 pH units in sodium pyrophosphate treated meat does not appreciably change the tenderness of the meat. No specific trends were noted relating the tenderness of meat to mineral components. Comparison of the effect of phosphates as a single unit indicated that phosphates significantly increased the tenderness at the 5% level. The pH values were also significantly different from the untreated samples in both the citrate- and phosphate- treated samples.

The change in pH caused by the addition of citrate and phosphate could possibly influence the solubility of muscle protein, the ionic equilibrium and other chemical processes. It should be noted that dibasic sodium phosphate when added to meat changed the pH by approximately 0.4 pH units and that this caused little or no effect on the distribution of calcium in the muscle.

The investigation of the distribution of calcium in meat samples after different chemical treatments indicated that calcium was removed from its binding with insoluble protein by the complexing action of the citrate and phosphate. Of the mineral additives, mono- and dibasic sodium phosphate showed little or no calcium complexing ability. Sodium citrate and sodium pyrophosphate showed some complexing ability whereas sodium tripolyphosphate, sodium tetraphosphate and sodium hexametaphosphate proved to be the most effective complexing agents. These findings agree with

those of Batra and deMan (1965), who showed, by measuring free calcium ions in mixtures of orthophosphates, polyphosphates, and citrate salts, that primary and secondary orthophosphates have no complexing ability; on the other hand, sodium citrate, sodium hexametaphosphate, sodium tripolyphosphate, and sodium tetraphosphate were effective calcium complexing agents.

The study of the distribution of magnesium in meat samples showed that the magnesium equilibrium is unaffected by the presence of added citrate and phosphates.

This investigation was concerned with the concentration of certain inorganic elements in meat, their relative degree of binding and the relationship of muscle components to changes in tenderness. Despite the detailed nature of the chemical and physical analyses used in this study, there was no apparent relation between tenderness and individual components of beef muscle. This indicates that tenderness of meat is not related to a single factor in the mineral composition but possibly to a combination of the variables determined. The combined relationship of several of the mineral ions could be of significance.

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APPENDIX

Table A-1. Classification of Phosphates Used in the Analysis.

Meta		Pyro	Ortho
	polyphosphates ($M_{n+2}P_nO_{3n+1}$)		
Ring	long chain		
(sodium hexametaphosphate)	(sodium tetraphosphate)	(sodium pyrophosphate)	(monobasic sodium phosphate)
	(sodium tripolyphosphate)		(dibasic sodium phosphate)

Phosphates may be defined as those phosphorus compounds in the anions of which each atom of phosphorus is surrounded by four oxygen atoms arranged at the corners of a tetrahedron. By sharing oxygen atoms between tetrahedron chains, rings and branched polymers of interconnected phosphate tetrahedra can be produced. The simple phosphate monomeric unit is termed orthophosphates. The simple -P-O-P- chains are called polyphosphates and the ring systems are termed metaphosphates. Sometimes dipoly-, tripoly- and tetrapolyphosphates are termed di-, tri- or tetraphosphates.

Table A-2. Description and composition of phosphate and citrate used in the experiment.

Compound	Chemical Formula	Molecular Weight	Phosphorus inorganic or citrate	Phosphorus or citrate as percent molecular weight (%).
(1) Orthophosphate (a) monobasic sodium phosphate	$NaH_2PO_4.H_2O$	138	30.97	22.44
(b) dibasic sodium phosphate	Na_2HPO_4	142	30.97	21.81
(2) Sodium Pyrophosphate	$Na_4P_2O_7.10H_2O$	446	61.95	12.89
(3) Sodium tripoly-phosphate	$Na_5P_3O_{10}$	368	92.92	25.25
(4) Sodium tetra-phosphate	$Na_6P_4O_3$	470	123.90	26.36
(5) Sodium hexa-metaphosphate	$(NaPO_3)_6$	612	185.83	30.37
(6) Sodium citrate	$Na_3C_6H_5O_7.2H_2O$	294	189.03	64.25